

Laser's Effect on Bone and Cartilage Change Induced by Joint Immobilization: An Experiment With Animal Model

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Objective: Influence of low-level (810nm, Ga-Al-As semiconductor) laser on bone and cartilage during joint immobilization was examined with rats' knee model.

Materials and Methods: The hind limbs of 42 young Wistar rats were operated on in order to immobilize the knee joint. One week after operation they were assigned to three groups; irradiance 3.9W/cm², 5.8W/cm², and sham treatment. After 6 times of treatment for another 2 weeks both hind legs were prepared for 1) indentation of the articular surface of the knee (stiffness and loss tangent), and for 2) dual energy X-ray absorptiometry (bone mineral density) of the focused regions.

Results and Conclusions: The indentation test revealed preservation of articular cartilage stiffness with 3.9 and 5.8W/cm² therapy. Soft laser treatment has a possibility for prevention of biomechanical changes by immobilization. *Lasers Surg. Med.* 21:480–484, 1997. © 1997 Wiley-Liss, Inc.

Key words: bone density; disuse osteocartilagenous change; joint immobilization; laser biostimulation; viscoelastic measurement

INTRODUCTION

Low-level laser therapy (low-energy or "cold" laser, low-power or "soft" laser) has been used since the late 1960s, when the early research projects started, especially in Eastern countries [1]. Many investigations were conducted which focused on the repairing aspect of laser biostimulation for musculoskeletal system and skin [2–4] as well as pain and nerve function control. The mechanism of the effectiveness of soft laser therapy is still unclear in spite of more than 20 years of clinical use and investigation.

From our previous experiment using the same immobilized rat's model as the present one [5], we know that disuse or lack of mechanical stress dramatically impairs bone metabolism next to the joint in young animals. With that observation, the present study is designed to evaluate the

effect of low-energy laser on remodeling aspect of bone and cartilage during immobilization.

MATERIALS AND METHODS

Operative and Therapeutic Procedure

Forty-two Wistar-Imamichi male rats (9 weeks old, 300–350g) were anesthetized with pentobarbital sodium (40mg/kg). Subcutaneous femorotibial ligature according to Wilson and Dahners [6] with non-absorbable suture (#0 Ethibond, Ethicon Inc., Tokyo, Japan) on one leg of the rats

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kept the knee joint in approximately 150° of flexion. The needle without a suture was passed on the opposite side as a sham operation.

All rats were kept freely for 1 week after operation, and then randomly assigned to three groups; sham, 60mW, and 40mW. During treatment the animals were kept in a wooden box and were unable to stir. Shaving the skin over the lateral joint space of the immobilized knee, the tip of a laser probe was attached directly to the skin through an open window of the box.

Laser Therapy Condition

Treatment procedure was planned on the basis of a preliminary study with the same materials using 10mW (irradiance 0.6W/cm²) and cumulative doses 15 and 45 J for another 1 and 3 weeks. Because no statistically significant difference was noted between the sham-treated animals and free animals which were not restrained in a box from the preliminary study, we used three groups in this experiment.

The laser (JQ305, Nihon Infrared Industry, Tokyo, Japan) used was a Ga-Al-As semiconductor type, 810 nm in wavelength and up to 100mW tuneable intensity. The beam geometry was rectangular and the spot area was 1.04 mm². As this device was unable to operate continuously for more than 1 min, the procedure was done repeatedly for a total of 3 min/day, and provided 6 times (every 2 days on week days) for another 2 weeks. The incident energy density was 3.9W/cm² for 40mW intensity, and 5.8W/cm² for 60mW intensity. The cumulative total doses resulted in 43J, and 65J respectively.

Preparation for Specimen

Each rat in the groups was killed and both hind legs were harvested. After the muscles of the thigh and lower legs were removed, the disarticulation through the ankle was carried out. The specimens were wrapped with wet gauze and stored in a plastic bag at -80°C. The wrapped specimens were thawed at room temperature, and opening of the knee joint was done just before mechanical testing to make it into two pieces; the femur and the tibia.

Biomechanical Testing

Using a viscoelastic spectrometer (DDV-VMF, Orientec, Tokyo, Japan) the indentation method was carried out first. A chucking-type holding device gripped the specimen in an upward position. An indenter with 2mm diameter pro-

vided a sinusoidal vibrating load of amplitude 2 N over the preload 3 N ($F_0 = 3\text{N}$, $F_1 = 2\text{N}$) on the articular surface.

$$\text{Load : } F = F_0 + F_1 \sin \omega t$$

$$\text{Deformation : } D = D_0 + D_1 \sin (\omega t - \delta)$$

where ω : angular frequency, δ : phase-lag

This dynamic test indicated a structural stiffness (F_1 / D_1 ; amplitude of load divided by amplitude of displacement) which represented elastic resistance of the osteocartilagenous composite, and a loss tangent (tangent of phase-lag δ) which related to the amount of dissipation of energy. The linear condition between the load and the deformation during the experiment was confirmed on an oscilloscope (V-550B, Hitachi Denshi, Tokyo, Japan). Considering the linear condition of vibration response and its reproducibility by the capability of the testing machine, frequencies of 11Hz and 35 Hz were selected. Four measurement sites were selected at both the lowest point of the medial and lateral femoral condyles, and both the center of the medial and lateral tibia plateaus around the knee joint.

Bone Mineral Density (BMD) Measurement

With a dual energy X-ray absorptiometry (DCS-600, Aloca, Co., Tokyo, Japan), a region of interest (ROI) with a 3mm width was selected at the four sites (midshaft and distal metaphysis of the femur, proximal metaphysis and midshaft of the tibia) using a 2-level (27KeV, 53KeV) monochromatic X-ray beam.

Statistical Analysis

Statistical analyses according to analysis of variance (one way ANOVA) were performed for each group. Student's *t*-test was also used to compare the immobilized, experimental limb and the control, free limb. The analysis was carried out using a computer software program (Statview 2.1J, Abacus Concepts, Berkeley, U.S.A.).

RESULTS

Status of the Animals

All rats that were kept showed good recovery from the surgical intervention by the following day. Of the original 42 rats, however, thirteen rats were excluded from the analysis because of little weight gain due to infection during the period. Body weight gain of 3 groups are as follows:

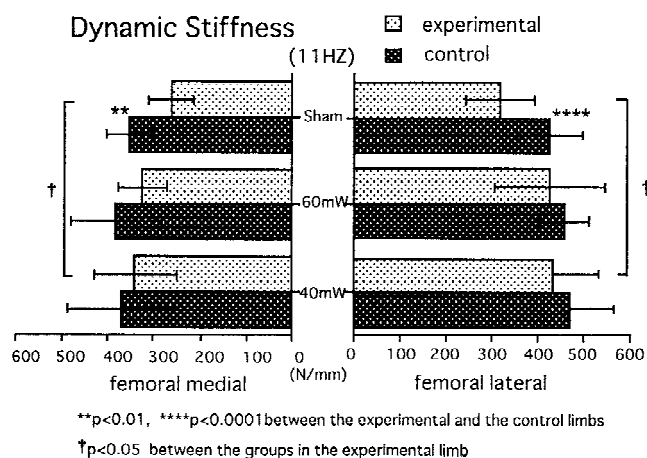


Fig. 1. Graph of the dynamic stiffness of the femoral specimen (11Hz).

77.6 ± 18.2g (n = 10) for sham, 75.6 ± 16.7g (n = 9) for 60mW, and 70.7 ± 10.2g (n = 10) for 40mW. Many rats had periosteal reaction at the anterior margin of the tibial shaft due to friction by the ligature.

Results of Analysis

Soft laser treatment revealed statistically significant differences in the stiffness of biomechanical analysis. At the femoral condyles, significant difference was found between the sham and laser 40 mW, (Figure 1) and at the tibial plateau differences were also found between the sham and laser 40mW, and the sham and laser 60mW. (Figure 2) The phase-lag showed partial influence by laser treatment only at the tibial lateral plateau (Figure 3). There was a tendency for the degree of the difference between the experimental, immobilized limbs and the control, free limbs to decrease under the influence of lasers. The experimental limb always showed a significantly lower BMD than the free limb, except in the tibial shaft analysis. There were a few significant changes of BMD in the control limb among treated groups at the femoral metaphysis and at the tibial shaft. The explanation for these remains unclear. However, the BMD from the experimental limb indicated no obvious effect by laser stimulation in these conditions. (Table 1)

DISCUSSION

Although high-energy laser therapy for surgery and hemostasis is well known, the effect of low-energy laser, which usually means less than 60–100mW power intensity and is regarded as

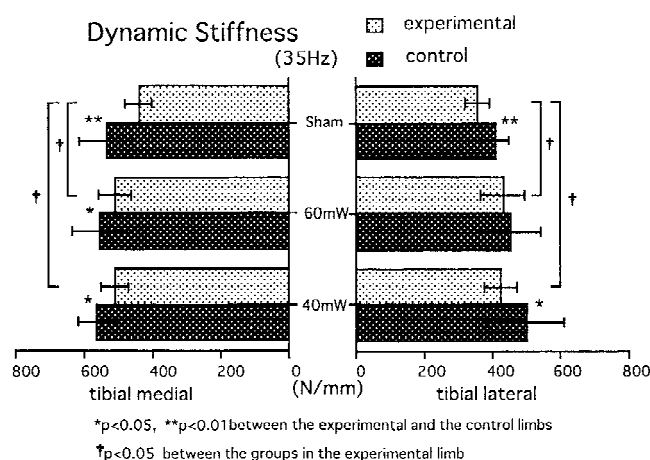


Fig. 2. Graph of the dynamic stiffness of the tibial specimen (35Hz).

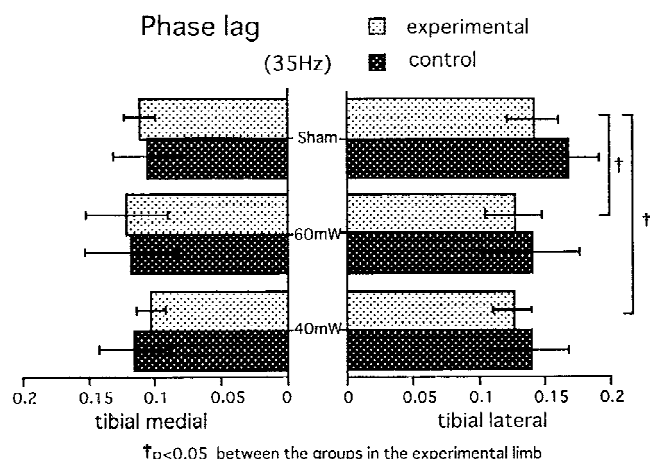


Fig. 3. Graph of the the phase-lag of the tibial specimen (35Hz).

showing a non-thermal effect, still remains surrounded by skepticism [7]. Observed and reported effects cover alteration of nerve function, acceleration of wound healing, treatment of musculoskeletal symptoms, and for pain control [2,3,8]. Regarding in vitro experiments, widespread effects were revealed on cellular functions [7,9]. Even in animal experiments, there are several reports on the action of lasers to enhance osteogenesis [10,11].

Stimulation Modality

From a physics point of view, the mode of soft laser is light stimulation [12], which consists of 3 aspects, i.e., monochromaticity (single frequency band), collimation (narrow, focused beam), and coherency (waves in phase). In biostimulative conditions in the in vivo experiment, the laser

TABLE 1. Bone Densitometry (g/cm²) at the Four Different Regions of Interest†

Group	Femoral shaft experimental limb	Control limb	Femoral distal experimental limb	Control limb	Tibial proximal experimental limb	Control limb	Tibial shaft experimental limb	Control limb
Sham	**0.109 ± 0.009	0.117 ± 0.008	**0.170 ± 0.009	***0.199 ± 0.014	***0.107 ± 0.011	0.137 ± 0.008	*0.134 ± 0.018	***0.118 ± 0.011
60mW	*0.110 ± 0.007	0.119 ± 0.005	**0.162 ± 0.012	0.193 ± 0.013	***0.111 ± 0.006	0.142 ± 0.009	0.133 ± 0.009	0.126 ± 0.009
40mW	*0.113 ± 0.007	0.120 ± 0.007	**0.162 ± 0.018	***0.184 ± 0.019	***0.109 ± 0.009	0.134 ± 0.011	0.135 ± 0.012	***0.131 ± 0.011

†mean ± standard deviation.

*P < 0.05, **P < 0.01, ***P < 0.0001 between the experimental and the control limbs, ****P < 0.05, *****P < 0.01 between the laser and the sham groups.

beam had lost its properties by tissue scattering and rapidly degrading collimation and coherency. As light energy decreases exponentially with depth, reaction may be less effective at the deeper tissue levels. An effect produced by soft laser may be not due to the unique quality of lasers but due to the effect of light. The effective doses in this experiment were 3.9–5.8 W/cm², and were much higher than those previously reported. Kana et al. [13] claim that treatment should be at intensities of about 40 to 50 mW/cm² with cumulative energies delivered of about 1–4 J/treatment. Parameters such as wavelength, treatment duration, energy density, and mode of treatment are critical. The concept of “biological window” is, therefore, present throughout nature including the soft laser.

Animal Model Choice

As a general tendency in the preliminary study the difference of properties between the medial and the lateral measurement sites was noted at the femur and the tibia. In the distal end of the femur the articular surface of the medial condyle showed less stiffness than that of the lateral condyle. In contrast to this, in the proximal end of the tibia, the lateral plateau had less stiffness than the medial plateau. The study also showed that the bones of the immobilized limb lost the trend of biomechanical parameters found in the free limb; in which the stiffness increased and the phase-lag decreased during time course. Though the differences between 11Hz and 35Hz were not obvious, the response of the stiffness and the phase lag was different [5]. And bone densitometry revealed increased BMD during observations at the shaft and the metaphysis except at the tibial shaft. In interpreting the present results we have to consider site and time differences of observed parameters from these young growing animals.

Restraint of rat in a holding box might be a great stress for such small animals and be a concern for this experimental condition. We found no differences of weight gain among the 3 groups. Growth and body weight gain are important factors of mechanical properties and bone density in rats [5,14].

Bone Metabolism Changes

The indentation test has been used to evaluate joint cartilage degeneration which is associated with mechanical changes of the material, especially stiffness [15]. Plaster fixation, bed rest, and the other weightless states all result in cal-

cium loss and bone atrophy if continued for a few weeks [16]. However the pathogenesis of immobilization-induced bone loss is still unclear. Decreased bone formation and increased bone resorption have been implicated in rats and rabbits, and these changes were more pronounced in trabecular bones [17]. Akeson et al. [18] summarized the effect of joint immobilization in their review article. They state that synovial joints require the stimulation of physical activity for homeostasis; i.e., the ability to retain the biochemical matrix composition, and the biomechanical characteristics associated with normal joints.

Mechanisms of Biostimulation

Recently many biophysical influences e.g., control of growth, repair and remodeling in cellular systems gained researchers' attention. Mechanical loading has a long clinical history [19,20], especially cyclical strain [21], and ultrasound [22], low-power laser [11], electromagnetic field [23,24] are also reported to have effects on various aspects of repair and remodeling in musculoskeletal systems. The authors conducted an animal experiment which revealed the effect of electricity on joint immobilization with the same experimental condition as that in the present one [25]. Moreover, some physical energies may produce some biological effects [7,26].

Further accumulation of data will be needed to determine the optimal dosage and treatment schedule for low-level laser for prevention of bone and cartilage change by joint immobilization.

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